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(71) Applicant *(for all designated States except US)*: M.L. LABORATORIES PLC [GB/GB]; Rutherford Close, Wavertree Technology Park, Liverpool L13 1EJ (GB).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)* : DAVIES, Donald, Selwyn [GB/GB]; 7 Mynden Close, Beaconsfield, Buckinghamshire HP9 2AU (GB). WEBER, Jonathan, Norden [GB/GB]; 50 St Paul's Road, London NW1 2QW (GB).

(74) Agent: DIBB LUPTON BROOMHEAD AND PRIOR; 117 The Headrow, Leeds LS1 5JX (GB).

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(54) Title: PHARMACEUTICAL COMPOSITION COMPRISING CD4 AND A POLYANIONIC ANTI-HIV AGENT AND USE THEREOF

(57) Abstract

The invention provides an agent against HIV and related viruses, in dosage unit form, comprising CD4 or a CD4-like material and a polyanionic anti-HIV agent, the content of CD4 or CD4-like material in the agent being less than the anti-virally effective dose of the CD4 or CD4-like material alone. It has been found that when the CD4 or CD4-like material is used together with a polyanionic anti-HIV agent, the combination is more effective against HIV infection than would be the case if the anti-viral effect was simply additive.

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+ DESIGNATIONS OF "SU"

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PHARMACEUTICAL COMPOSITION COMPRISING CD4 AND A POLYANIONIC ANTI-HIV AGENT AND USE THEREOF

This invention relates to pharmaceutically active compositions and to their use as agents against human immunodeficiency virus and related viruses.

The HIV infection cycle includes a number of steps, each of these steps being a possible target for the use of chemotherapy to hinder the infection. The first step in the infection cycle is the attachment of the HIV virus to the host cell. The main receptor for the virus is believed to be the cell surface glycoprotein, designated CD4, which is present in some T-lymphocytes and in some macrophages. Much attention has therefore been given to identifying agents which are capable of blocking attachment of the virus to CD4.

It is believed that the HIV virus attaches itself to CD4 by means of interaction between CD4 and the surface glycoprotein, gp120, of the virus. It has been found that soluble recombinant CD4 (srCD4), made by genetic engineering, can block attachment of HIV virus to cellular CD4, thereby inhibiting HIV infection in vitro. Therefore, it has appeared that HIV infection might be preventable by administration of srCD4. Other CD4-like materials, capable of binding to gp120, such as CD4-IgFc immunoadhesins, CD4 V1-V2 domains, and CD4-derived peptides, have also been proposed as anti-HIV agents. However, the performance

of CD4 and CD4-like materials in preventing the HIV virus from binding to cells has until now been disappointing, a much higher concentration of the agent being necessary than had been hoped.

Other anti-HIV agents which block attachment of the virus to cells are a number of polyanionic compounds which include Evans Blue, aurintricarboxylic acid (ATA), suramin, and certain sulphated polysaccharides. It might have been thought that if CD4 and such polyanionic anti-HIV agents were used simultaneously, the total anti-HIV activity would at best be the sum of their separate activities.

However, we have now found that when CD4 (which is used here and below, except where otherwise indicated, to include both CD4 and CD4-like materials) and a polyanionic anti-HIV agent, such as sulphated polysaccharide, are used together, they are much more effective against HIV infection than would be the case if the anti-viral effect was simply additive.

Accordingly, the invention provides an agent against HIV and related viruses, in dosage unit form, comprising CD4 or a CD4-like material and a polyanionic anti-HIV agent the content of CD4 and CD4-like material in the agent being less than the anti-virally effective dose of the CD4 or CD4-like material alone.

The agent according to the invention may contain substantially

less CD4 than the anti-virally effective dosage of CD4 alone, i.e. in the absence of a polyanionic anti-HIV agent. The reduction in the amount of CD4 required to achieve the same anti-viral effect as for CD4 alone may be such that less than one-tenth (or, in favourable cases, less than one-hundredth) of the amount of CD4 is needed. Accordingly, the invention offers the possibility of using CD4 as a component of an anti-HIV agent in a reduced and therefore safer and more economical amount than has previously been feasible.

The agent according to the invention may also contain less than the normally effective dose of the polyanionic compound. This is advantageous because many of the known polyanionic anti-HIV agents are significantly toxic. For example, sulphated polysaccharides have anti-coagulant activity. When the polyanionic anti-HIV agent is a sulphated polysaccharide, it may be present in the compositions of the invention in an amount which is less than that which would be required in an anti-HIV agent containing only the sulphated polysaccharide.

Consequently, the compositions of the invention which contain sulphated polysaccharides may have a lower level of anti-coagulant activity attributable to the sulphated polysaccharide content than those previously known.

The polyanionic anti-HIV agents used in the present invention are

preferably sulphated polysaccharides. They include, for example, dextran sulphate, pentosan polysulphate, fuccidan, and dextrin sulphate. Other sulphated polysaccharides having anti-HIV activity (see, for example, EP specifications No's 240,098 and 293,826) may also be used. Preferably, the sulphated polysaccharide contains at least one sulphate group per saccharide unit.

In therapeutic use, the anti-HIV agent of the invention may be administered enterally (including orally) or parenterally (including intravenously). However, administration via the peritoneum may be more effective in that it results in entry of at least some of the anti-HIV agent directly into the lymphatic system, within which system viral replication may be extensive.

The invention also provides the use of the agent described above against HIV-1 and relates viruses, the agent preferably being administered peritoneally.

Further, the invention provides a pharmaceutical composition containing the anti-HIV agent of the invention together with an inert carrier or diluent and the agent of the invention for use in the manufacture of a pharmaceutical composition against HIV-1 and related viruses.

The invention additionally provides a method of treatment of a

human or animal subject carrying the HIV-1 virus or a related virus, comprising administering to the subject a pharmaceutically effective amount of the agent of the invention.

The CD4 or CD4-like material and the polyanionic anti-HIV agent may be administered to a subject one after the other, in any order although preferably with the CD4 or CD4-like material being administered before the polyanionic agent, when used against HIV-1 or a related virus.

The invention thus provides CD4 or a CD4-like material, of an amount less than its usual anti-virally effective dose, and a polyanionic anti-HIV agent, for use in a method of treatment of a human or animal subject carrying the HIV-1 virus or a related virus in whic the CD4 or CD4-like material are administered to the subject one after the other.

The following example illustrates the synergistic action of dextrin sulphate with soluble recombinant CD4 (srCD4). The dextrin sulphate was produced by sulphation of a dextrin of weight average molecular weight of about 20,000 daltons, using a sulphur trioxide/trimethylamine complex, the degree of substitution being approximately one sulphate group per glucose unit. The srCD4 was produced in a baculo virus system and was purchased from American Biotechnology Inc.

Example 1

In a duplicated experiment, 2-fold dilutions of soluble recombinant CD4, to yield final concentrations of 6 ug/ml down to 0.02 ug/ml, were incubated with 2.5 x 103 TCID (tissue culture infection dose) of HIV-1 virus supernatant (HTLV-IIIb strain, Gallo, 1984) at 37°C for 1 hour. 2-fold dilutions of dextrin sulphate to yield final concentrations from 10 ug/ml down to 0.625 ug/ml were then added to the T-cell line, M8166, at a density of 2.5 x 10^5/ml and incubated at 37°C for 1 hour. The srCD4/HIV-1 was then added to the dextrin sulphate pre-treated M8166 cells, and the plates read at 48 - 72 hours for the presence of syncytia, shown in the table below by +.

In the absence of dextrin sulphate, srCD4 inhibited HIV-1 infection of M8166 cells at 6 ug/ml (final concentration). In the absence of srCD4, dextrin sulphate inhibited HIV-1 infection of M8166 cells at 5-10 ug/ml. When both drugs were present, 2.5 - 5.0 ug/ml of dextrin sulphate inhibited HIV-1 infection in the presence of srCD4 at a concentration of 0.05 - 0.09 ug/ml. This represents a 10^2 reduction in the quantity of srCD4 required to prevent HIV-1 infection, and shows that the effect of the two drugs together is synergistic rather than simply additive.

FIRST EXPERIMENT

Dextrin Sulphate (ug/ml)	srCD4 (ug/ml)									
	6	3	1.5	0.75	0.375	0.18	0.09	0.05	0.02	(
5	-	-	-	-	-	-	-	-	-	
2.5	-	-	-	-	-	-	-	-	+	
1.25	-	-	-	-	+	+	+	+	+	
0.625	-	-	+	+	+	+	+	+	+	
0	-	+	+	+	+	+	+	+	+	

SECOND EXPERIMENT

Dextrin Sulphate (ug/ml)	srCD4 (ug/ml)									
	6	3	1.5	0.75	0.375	0.18	0.09	0.05	0.02	C
10	-	-	-	-	-	-	-	-	-	
5	-	-	-	-	-	-	-	+	+	+
4	-	-	-	-	+	+	+	+	+	+
3	-	-	+	+	+	+	+	+	+	+
2	-	+	+	+	+	+	+	+	+	+
1	-	+	+	+	+	+	+	+	+	+
0	-	+	+	+	+	+	+	+	+	+

METHOD

A srCD4 diluted into 96 well tray.

Add 2.5 x 10^3 TCID IIIb.

Incubate at 37oC for 1 hour.

B Dextrin sulphate diluted into another well tray.

Add M8166 (2.5 x 10^5/ml).

Incubate at 37°C for 1 hour.

Then add A to B.

Read at 2 - 3 days when positive controls show >95% syncytia.

CLAIMS

1. An agent against HIV and related viruses, in dosage unit form, comprising CD4 or a CD4-like material and a polyanionic anti-HIV agent, the content of CD4 or CD4-like material in the agent being less than the anti-virally effective dose of the CD4 or CD4-like material alone.

2. The agent of Claim 1 wherein the content of CD4 or CD4-like material is less than one-tenth of the anti-virally effective dose of the Cd4 or CD4-like material alone.

3. The agent of Claim 1 wherein the content of CD4 or CD4-like material is less than one-hundredth of the anti-virally effective dose of the Cd4 or CD4-like material alone.

4. The agent of any of Claims 1 to 3 wherein the content of said polyanionic anti-HIV agent is less than the anti-virally effective dose of the polyanionic anti-HIV agent alone.

5. The agent of any of Claims 1 to 4 wherein said polyanionic anti-HIV agent is a sulphated polysaccharide.

6. The agent of Claim 5 wherein the sulphated polysaccharide is dextrin sulphate.

7. The agent of Claim 5 or 6 wherein said sulphated polysaccharide contains at least one sulphate group per saccharide unit.

8. The use of an agent according to any preceding claim against HIV-1 and related viruses.

9. The use of an agent, as claimed in Claim 8, wherein the agent is administered peritoneally.

10. The agent of any of Claims 1 to 7, adapted for intraperitoneal administration.

11. The agent of any of Claims 1 to 7, for use in the manufacture of a pharmaceutical composition against HIV-1 and related viruses.

12. A pharmaceutical composition containing the agent of any of Claims 1 to 7 and an inert carrier or diluent.

13. A method of treatment of a human or animal subject carrying the HIV-1 virus or a related virus, comprising administering to the subject a pharmaceutically effective amount of the agent of any of Claims 1 to 7.

14. CD4 or CD4-like material, of an amount less than its usual anti-virally effective dose, and a polyanionic anti-HIV agent, for use in a method of treatment of a human or animal subject carrying the HIV-1 virus or a related virus in which the CD4 or CD4-like material and the polyanionic agent are administered to the subject one after the other, in any order.

15. A method of treatment of a human or animal subject carrying the HIV-1 virus or a related virus, comprising administering to the subject, one after the other but in any order, CD4 or a CD4-like material, of an amount less than its usual anti-virally effective dose, and a polyanionic anti-HIV agent.

16. A method according to Claim 15, wherein the CD4 or CD4-like material is administered before the polyanionic anti-HIV agent.

AMENDED CLAIMS

[received by the International Bureau on 12 March 1992 (12.03.92); original claims 5 and 6 cancelled; original claims 1-4 and 7-16 amended and renumbered accordingly (2 pages)]

1. An agent against HIV and related viruses, in dosage unit form, comprising CD4 or a CD4-like material and dextrin sulphate, the content of CD4 or CD4-like material in the agent being less than the anti-virally effective dose of the CD4 or CD4-like material alone.

2. The agent of Claim 1 wherein the content of CD4 or CD4-like material is less than one-tenth of the anti-virally effective dose of the CD4 or CD4-like material alone.

3. The agent of Claim 1 wherein the content of CD4 or CD4-like material is less than one-hundredth of the anti-virally effective dose of the CD4 or CD4-like material alone.

4. The agent of any of Claims 1 to 3 wherein the content of said dextrin sulphate is less than the anti-virally effective dose of dextrin sulphate alone.

5. The agent of any one of the preceding claims wherein the dextrin sulphate contains at least one sulphate group per saccharide unit.

6. The use of an agent according to any preceding claim against HIV-1 and related viruses.

7. The use of an agent, as claimed in Claim 6, wherein the agent is administered peritoneally.

8. The agent of any of Claims 1 to 5, adapted for intraperitoneal administration.

9. The agent of any of Claims 1 to 5, for use in the manufacture of a pharmaceutical composition against HIV-1 and related viruses.

10. A pharmaceutical composition containing the agent of any of Claims 1 to 5 and an inert carrier or diluent.

11. A method of treatment of a human or animal subject carrying the HIV-1 virus or a related virus, comprising administering to the subject a pharmaceutically effective amount of the agent of any of Claims 1 to 5.

12. CD4 or CD4-like material, of an amount less than its usual anti-virally effective dose, and dextrin sulphate, for use in a method of treatment of a human or animal subject carrying the HIV-1 virus or a related virus, in which method the CD4 or CD4-like material and the dextrin sulphate are administered to the subject one after the other, in any order.

13. A method of treatment of a human or animal subject carrying the HIV-1 virus or a related virus, comprising administering to the subject, one after the other but in any order, CD4 or a CD4-like material, of an amount less than its usual anti-virally effective dose, and dextrin sulphate.

14. A method according to Claim 13, wherein the CD4 or CD4-like material is administered before the dextrin sulphate.

INTERNATIONAL SEARCH REPO[redacted]

International Ap[redacted]n No PCT/GB 91/01627

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl.5 A 61 K 37/02 // A 61 K 31/715

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl.5	A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
X	WO,A,9000596 (THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA) 25 January 1990, see page 12, lines 25-35; page 13, lines 1-20; pages 34-54; claims	1-7,10-12,14
Y	WO,A,8911860 (BIOGEN INC.) 14 December 1989, see page 5, lines 1-37; page 6, lines 1-28; pages 41-44; claims	1-7,10-12,14
Y	EP,A,0332952 (BASF AG) 20 September 1989, see the whole document	1-7,10-12,14
A	EP,A,0293826 (STICHTING REGA V.Z.W.) 7 December 1988, see the whole document	1-7,10-12,14

° Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
13-12-1991	16. 01. 92
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	Danielle van der Haas

Form PCT/ISA/210 (second sheet) (January 1985)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☒ OBSERVATION WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This International search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claim numbers *** because they relate to subject matter not required to be searched by this Authority, namely:

Pls. see Rule 39.1(iv) - PCT:
Method for treatment of the human or animal body by surgery or therapy, as well as diagnostic method.
*** Claims 8,9,13,15,16

2. ☐ Claim numbers because they relate to parts of the International application that do not comply with the prescribed requirements to such an extent that no meaningful International search can be carried out, specifically:

3. ☐ Claim numbers because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International search report covers all searchable claims of the International application

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this International search report covers only those claims of the International application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO. GB 9101627
SA 51588

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 13/01/92
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A- 9000596	25-01-90	EP-A- 0423240	24-04-91
WO-A- 8911860	14-12-89	AU-A- 3831889	05-01-90
		EP-A- 0378643	25-07-90
		JP-T- 2504641	27-12-90
EP-A- 0332952	20-09-89	DE-A- 3808353	21-09-89
		AU-A- 3122189	14-09-89
		JP-A- 1275532	06-11-89
EP-A- 0293826	07-12-88	JP-A- 1100127	18-04-89

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For more details about this annex : see Official Journal of the European Patent Office, No. 12/82